# Barotropic Phase Transitions and Pressure-induced Interdigitation on Bilayer Membrane of Dimyristoylphosphatidylcholine

Hayato Ichimori,† Hitoshi Matsuki, and Shoji Kaneshina*
*Department of Biological Science & Technology, Faculty of Engineering, The University of Tokushima, Minamijosanjima, Tokushima 770*
†*Anan National College of Technology, Minobayashi, Anan, Tokushima 774*

(Received September 16, 1997; CL-970718)

The thermotropic and barotropic phase transitions of dimyristoylphosphatidylcholine (DMPC) multilamellar vesicle have been studied. Both temperatures of the main transition and pretransition were elevated by pressure. A pressure-induced interdigitation of DMPC bilayer membrane was observed at high pressures above 300 MPa.

Phospholipid vesicles have been used most extensively as biomembrane models. The multilamellar vesicle of dimyristoylphosphatidylcholine (DMPC), as well as dipalmitoylphosphatidylcholine (DPPC), is well known to undergo the pretransition from the lamellar gel ($L_{\beta}'$) phase to the ripple gel ($P_{\beta}'$) phase, and succeedingly the main transition from the $P_{\beta}'$ phase to the liquid crystal ($L_{\alpha}$) phase. The temperatures of the main transition and pretransition for DMPC[1] are known to be 23.9 ℃ and 14.3 ℃, respectively. Pressure studies on the phase behavior of DMPC bilayer membranes have been reported by various physical techniques including Raman spectroscopy,[2,3] adiabatic compression,[4] fluorescence,[5-7] neutron diffraction,[8,9] light transmittance[10,11] and densitometry.[12] All of these studies have revealed that the main transition temperature increases linearly with an increase in pressure and the values of $dT/dP$ for the main transition lie between 0.201 and 0.240 K $MPa^{-1}$. On the other hand, a few studies[2,7,10] have been reported on the pressure-dependence of the pretransition temperature, which are not consistent with each other. Moreover, the pressure-induced, interdigitated gel ($L_{\beta}I$) phase has not been observed yet in the DMPC bilayer membrane although the interdigitation of DPPC bilayer membrane has been already observed under high pressures.[8,9,13] The present study demonstrates the temperature-pressure phase diagram of DMPC multilamellar vesicle and reveals the existence of the pressure-induced $L_{\beta}I$ phase in the bilayer membrane.

Synthetic DMPC, 1,2-ditetradecanoyl-*sn*-glycero-3-phosphocholine, was obtained from Sigma. DMPC multilamellar vesicle was prepared by suspending DMPC in water at 2.0 mmol $kg^{-1}$, using a Branson model 185 Sonifier at a temperature several degrees above the main phase-transition.

The phase transitions under high pressures were observed by two kinds of optical methods. One is the observation of isothermal barotropic phase transitions and the other is the isobaric thermotropic phase transitions. The general arrangement of the high pressure apparatus has been described in detail previously.[11,14] The sudden change in transmittance accompanying the phase transition was followed at 560 nm. In addition to the optical observation, the phase transitions of DMPC bilayer membrane under ambient pressure were observed by a Micro Cal MCS high-sensitivity differential scanning calorimeter (DSC). The heating rate was 0.75 K $min^{-1}$.

An example of the thermotropic phase-transition measurements for DMPC bilayer membrane is shown in Figure 1. The DSC thermogram of heating scan at ambient pressure showed two kinds of endothermic transitions (curve 1 in Figure 1). Higher-temperature transition can be assigned as the main transition from the $P_{\beta}'$ phase to the $L_{\alpha}$ phase. On the other hand, lower-temperature transition can be assigned as the pretransition from the $L_{\beta}'$ phase to the $P_{\beta}'$ phase. The light transmittance also changed clearly at two transition temperatures (curve 2 in Figure 1). Two transition temperatures by both methods were in good agreement with each other. The temperatures of the main transition and pretransition of DMPC bilayer membrane were 24.0 ℃ and 13.9 ℃, respectively, which are in good agreement with previously published data.[1] Both temperatures of the main transition and pretransition increased with an increase in pressure. (curve 3 in Figure 1). A difference in temperature between two transitions became wide as the pressure increases. At the pressure higher than 300 MPa, we observed a new pressure-induced phase transition (curve 4 in Figure 1). It has been observed for DPPC[14] and dihexadecylphosphatidylcholine (DHPC)[14,15] bilayer membranes that the transition from the $L_{\beta}I$ phase to the $P_{\beta}'$ phase is accompanied with an increase in

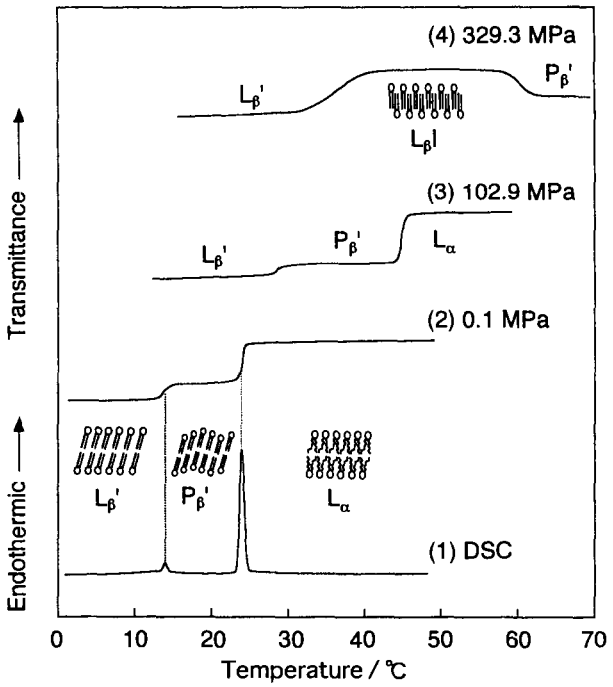


**Figure 1.** Isobaric thermotropic phase transitions of DMPC bilayer membrane. The main transition and pretransition by the methods of (1) DSC and (2) light transmittance at 0.1 MPa and (3) at 102.9 MPa. A pressure-induced interdigitation by the optical method (4) at 329.3 MPa.

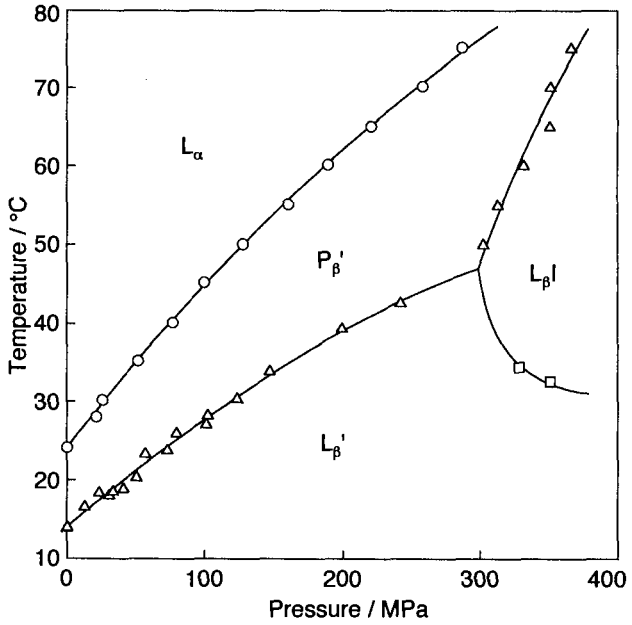


**Figure 2.** Phase diagram of DMPC bilayer membranes. The concentration of DMPC was 2.0 mmol $kg^{-1}$. Phase transitions: (O) $P_\beta' \rightarrow L_\alpha$, (Δ) $L_\beta' \rightarrow P_\beta'$ or $L_\beta I \rightarrow P_\beta'$, (□) $L_\beta' \rightarrow L_\beta I$.

turbidity. More direct evidence for interdigitaition has been observed from the spacing measurements by neutron diffraction[8,9] or X-ray diffraction[16] method. The phase transitions by the methods of light transmittance and X-ray diffraction were in good agreement with each other. The present new phase can be assigned as the $L_\beta I$ phase from analogy to the previous observation for DPPC[14] and DHPC.[14,15]

The temperature ($T$) - pressure ($P$) phase diagram of DMPC bilayer membrane is shown in Figure 2. The temperatures of the main transition and pretransition increase with an increase in pressure. The $T$-$P$ curves for the main transition and pretransition are slightly convex upward. The slopes of the phase boundary at ambient pressure were 0.212 K $MPa^{-1}$ for the main transition and 0.130 K $MPa^{-1}$ for the pretransition. The main transition of DMPC bilayer membrane under high pressure has been reported by several authors.[2-11] The previous values of $dT/dP$ for the main transition lie between 0.20 and 0.24 K $MPa^{-1}$ and the majority of the values are 0.22 K $MPa^{-1}$, which is in good agreement with the present result. Regarding the effect of pressure on the pretransition temperature, a few researchers have reported the values of $dT/dP$ to be 0.16,[2] 0.18,[7] and 0.12[10] K $MPa^{-1}$, which are not consistent with each other. A detailed pressure study has been reported by Prasad and coworkers.[10] Their result, 0.12 K $MPa^{-1}$ is comparable to the present value, 0.13 K $MPa^{-1}$.

The volume changes ($\Delta V$) associated with the transitions were calculated from the Clapeyron-Clausius equation,

$$dT/dP = T\Delta V/\Delta H.$$

The enthalpy changes ($\Delta H$) for the transitions were determined by the DSC measurements. The values of 24.7 kJ $mol^{-1}$ for the main transition and 4.6 kJ $mol^{-1}$ for the pretransition were employed. The values of $\Delta V$, calculated from the thermodynamic equation, were 17.6 $cm^3$ $mol^{-1}$ for the main transition and 2.1 $cm^3$ $mol^{-1}$ for the pretransition. The value of $\Delta V$ for the main transition is in good agreement with the previous value determined directly by a densitometry,[12,17,18] 17.2 $cm^3$ $mol^{-1}$.

As is seen from Figure 2, a pressure-induced interdigitated gel phase, in which the hydrocarbon chains from apposing monolayers become interdigitated with the chains, was observed at pressures above 300 MPa. A triple point on the phase diagram among $P_\beta'$, $L_\beta'$ and $L_\beta I$ phases was found at 300 MPa and 47 °C. A similar triple point for DPPC bilayer membrane has been found at 100 MPa and 45 °C.[13] With respect to distearoylphosphatidylcholine (DSPC),[8] interdigitaition of bilayer membrane has been observed at pressures above 80 MPa although a triple point has not been determined. As far as we know the pressure-induced inerdigitation of DMPC bilayer membrane has not been observed yet. Braganza and Worcester[8] have been predicted that hydrocarbon chain interdigitation may occur for DMPC bilayer membrane above 220 MPa from the neutron diffraction study on the pressure-induced interdigitation for DPPC and DSPC bilayer membranes. However, Winter and Pilgrim[9] have not observed the interdigitation in DMPC bilayer membrane up to a pressure of 300 MPa at 40 °C. The present results suggest that the interdigitation occurs for DMPC above the pressure of 310 MPa at 40 °C. The pressure at the triple point can be regarded as the minimum pressure for the interdigitation of lipid bilayer membranes. The pressure studies for a series of DMPC, DPPC and DSPC membranes demonstrate that the minimum pressure of interdigitation decreases with increasing acyl-chain length in a manner of nonlinear relation. This is clearly a consequence of the larger energy gain from van der Waals' interactions and the expected larger decrease in hydrocarbon volume per mole of lipid when longer chains interdigitate, so that it becomes favorable for longer chains to interdigitate at lower pressures.

## References and Notes

1 R. N. A. H. Lewis, N. Mak, and R. N. McElhaney, *Biochemistry*, **26**, 6118 (1987).
2 P. T. T. Wong, W. F. Murphy, and H. H. Mantsch, *J. Chem. Phys.*, **76**, 5230 (1982).
3 P. T. T. Wong and H. H. Mantsch, *Biochemistry*, **24**, 4091 (1985).
4 N. D. Russell and P. J. Collings, *J. Chem. Phys.*, **77**, 5766 (1981).
5 P. L. G. Chong and G. Weber, *Biochemisyry*, **22**, 5544 (1983).
6 J. R. Lakowicz and R. B. Thompson, *Biochim. Biophys. Acta*, **732**, 359 (1983).
7 W. D. Turley and H. W. Offen, *J. Phys. Chem.*, **89**, 3962 (1985).
8 L. F. Braganza and D. L. Worcester, *Biochemistry*, **25**, 2591 (1986).
9 R. Winter and W. C. Pilgrim, *Ber. Bunsenges. Phys. Chem.*, **93**, 708 (1989).
10 S. K. Prasad, R. Shashidhar, B. P. Gaber, and S. C. Chandrasekhar, *Chem. Phys. Lipids*, **43**, 227 (1987).
11 S. Kaneshina, K. Tamura, T. Isaka, and H. Matsuki, in "High Pressure Liquids and Solutions," ed by Y. Taniguchi, M. Senoo, and K. Hara, Elsevier Science B. V., Amsterdam (1994), p. 95.
12 N. Vennemann, M. D. Lechner, T. Henkel, and W. Knoll, *Ber. Bunsenges. Phys. Chem.*, **90**, 888 (1986).
13 S. Kaneshina, K. Tamura, H. Kawakami, and H. Matsuki, *Chem. Lett.*, **1992**, 1963.
14 S. Maruyama, H. Matsuki, H. Ichimori, and S. Kaneshina, *Chem. Phys. Lipids*, **82**, 125 (1996).
15 J. A. Veiro, P. Nambi, and E. S. Rowe, *Biochim. Biophys. Acta*, **943**, 108 (1988).
16 J. T. Kim, J. Mattai, and G. G. Shipley, *Biochemistry*, **26**, 6592 (1987).
17 D. A. Wilkinson and J. F. Nagle, *Biochemistry*, **20**, 187 (1981).
18 R. Koynova, A. Koumanov, and B. Tenchov, *Biochim. Biophys. Acta*, **1285**, 101 (1996).